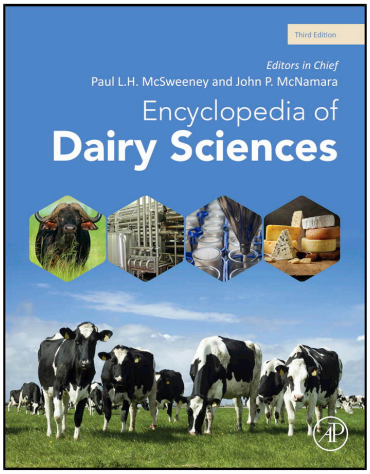

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Enzymes Indigenous to Milk: Lactoperoxidase

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Introduction

Lactoperoxidase (LPO) is an important indigenous milk enzyme because

- it exhibits antimicrobial activity,
- it is utilized for the preservation of milk quality,
- it is used as an index of the thermal history of milk – it is used as an index of super-high-temperature short-time (HTST) pasteurization,
- it is used as a commercial source of enzyme,
- it plays an important biological role in protecting the lactating mammary gland, and
- it causes nonenzymatic oxidation of unsaturated lipids, acting through its heme group.

LPO (EC 1.11.1.7) is a member of the peroxidase family, which includes a group of enzymes that are widely distributed in nature, having been found in plants and animals, including man. The peroxidase enzymes catalyze the oxidation of numerous organic and inorganic substrates by hydrogen peroxide:

$$2HA + H_2O_2 \rightarrow 2A + 2H_2O$$

where HA is an oxidizable substrate or a hydrogen donor, for example, aromatic amines, phenol, aromatic acids, or leuco dye.

LPO together with myeloperoxidase (MP), eosinophil peroxidase (EP), and thyroid peroxidase (TP) constitutes the mammalian peroxidase superfamily II, which is distinguished from the peroxidase superfamily I (includes enzymes from plants, fungi, and bacteria) in that the prosthetic heme group is covalently attached to the protein matrix. Most peroxidases, including LPO, contain ferriprotoporphyrin IX as a prosthetic group. A characteristic feature of hemoprotein peroxidases is their ability to exist in different oxidation states. There are five known enzyme intermediates. The major intermediates of LPO are ferric peroxidase (the native enzyme), compound I, compound II, compound III, and ferrous peroxidase.

LPO is an oxidoreductase secreted into milk; its primary role is to protect the mammary gland and the gut of infants against bacterial infections. LPO is found in the mammary, salivary, and lachrymal glands of all mammals tested so far and in their respective secretions, that is, milk, saliva, and tears. The peroxidases of the above-mentioned glands are chemically and immunologically similar. The LPO system acts as an antioxidant, thereby protecting mammalian cells against the highly reactive and damaging oxygen-derived species. Mammalian cells are not affected by the oxidation products of thiocyanate (SCN^-); the LPO system is not only atoxic to human cells but also protects these cells against the toxic effects of H_2O_2. Two principal forms occur, A and B, each of which exhibits microheterogeneity with regard to amide groups, glutamine and/or asparagine, and carbohydrate content, giving a total of 10 variants. There is no significant difference in the enzymatic activity of the various LPO fractions.

Physicochemical Properties of Lactoperoxidase

LPO was first recognized as early as 1881 by Arnold. Enriched preparations of LPO were obtained for the first time by fractional precipitation with $(NH_4)_2SO_4$ and the enzyme was subsequently isolated and crystallized by Theorell and Akeson in 1943. Improved isolation and characterization of the enzyme from rennet whey by salting out, displacement chromatography, and crystallization was reported in 1953 by Polis and Shmukler, which indicated that the enzyme was green in color and that it was contaminated with a red protein, now known as lactoferrin.

Since LPO is cationic at the pH of milk, it has been isolated and purified from milk or concentrated sweet (rennet) whey by cation-exchange chromatography using carboxymethyl cellulose at pH 5.1 or 5.7 and also using 0.05 mol L^{-1} phosphate buffer

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(pH 7.7) and a linear gradient of NaCl from 0 to 0.55 mol L^{-1}. LPO consists of a single polypeptide chain of 612 amino acid residues and shows homology with human MP (55%), EP (54%), and TP (45%). LPO is a basic protein and is highly structured, with 65% β-structure, 23% α-helical structure, and 12% unordered structure; it has a high isoelectric point of 9.6. The LPO enzyme is a 78 kDa glycoprotein with a heme group (protoheme 9) at its active site. The heme group in the catalytic center of the LPO molecule is a protoporphyrin IX; the heme is covalently bound to the polypeptide chain through two ester bonds, formed between the heme 1- and 5-position hydroxymethyl side chains and glutamate 375 and aspartate 275, respectively. LPO also binds Ca^{2+}, which stabilizes the molecular conformation of the enzyme and thus maintains its structural integrity. There is one calcium per iron atom and the protein has a high affinity for calcium. The iron content of LPO is 0.07%, corresponding to one iron atom per LPO molecule; iron is part of the heme group of the enzyme and is also associated with the carbohydrate chains of the enzyme. LPO has four or five carbohydrate chains, which constitute 10% of the 78 kDa mass. LPO has an absorbance maximum at 412 nm; its purity ratio is measured at A_{412}/A_{280} and it is approximately 0.95. The three-dimensional structure of LPO is in essence similar to that of MP found in the leukocytes of mastitic milk. An important difference is the more constraint heme pocket of LPO, being primarily responsible for the difference in the halide specificity of LPO and MP. At neutral pH, LPO oxidizes only iodide and thiocyanate; it can oxidize bromide only slightly but is unable to oxidize chloride.

Maximum LPO activity in milk is obtained at pH 6.0 using 2,2′-azino-bis-(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) as substrate. Since many of the reducing agents are chromogenic, a recommended method for measuring the LPO activity in milk is by using ABTS as a chromophore and measuring the absorbance at 412 nm. In milk, LPO is the second most abundant enzyme after xanthine oxidase, constituting ~0.5% of the total whey proteins and ~0.1% of total protein (30 mg L^{-1}).

LPO has high thermal stability in milk, whey, permeate, and buffer. Its destruction has been used as an index of the pasteurization efficiency of milk. LPO maintains its activity at low pasteurization temperatures for extended periods of time (63 °C, 30 min) and during HTST pasteurization (72 °C, 15 s), leaving sufficient activity (~70%) to catalyze the reactions between thiocyanate and hydrogen peroxide. Complete inactivation of LPO occurs by heating at 78 °C for 15 s. However, LPO loses its activity slowly at temperatures below 70 °C with a sharp decrease in its activity at 72 °C. LPO is denatured when heated at 80 °C for 2.5 s and it is possible that when LPO-activated milk is pasteurized, the LPO system can be reactivated to extend the shelf life of milk.

It seems that there is variation in the thermal inactivation of LPO in milk of different species. In camel milk for instance, LPO activity was found to be below detection limit after pasteurization (HTST) of the milk. However as opposed to cow milk, residual activity of the enzyme alkaline phosphatase was detected after heating camel milk at 72 °C for 5 min and, as a result, alkaline phosphatase is not suitable as marker for an effective pasteurization of camel milk. Thus, LPO is suggested as a suitable heat treatment indicator to verify an effective camel milk pasteurization. Kinetic and thermodynamic analysis of camel and bovine milk showed that camel milk lactoperoxidase is less heat-resistant and more sensitive to thermal denaturation as compared to bovine milk lactoperoxidase.

At low pH (5.3), LPO is less heat stable. The loss of calcium, which is responsible for the structural integrity of LPO enzyme, is the likely reason for the lower denaturation temperature of LPO at low pH. The greatest decrease in LPO activity at low concentrations (0.5 ppm) in the pH range 4.4–6.7 was 15% per 15 min at pH 5.4. At higher concentrations, >25 ppm, LPO did not lose activity. LPO is deactivated by storage at pH 3.

LPO is relatively stable against a number of proteolytic enzymes, for example, trypsin and thermolysin. LPO appears to be very sensitive to light in the presence of riboflavin and is inactivated by 55% in milk at 6000 lux after exposure for 4 h. Photochemical inactivation is irreversible but can be prevented by the addition of cysteine. LPO has a high tendency to adhere to surfaces, which leads to a decrease in activity. Structural prerequisites for the aggregation and adsorption are still unknown, but indications are that the LPO molecule is equipped for both ionic and hydrophobic interactions.

Variations in enzyme level are influenced by estrus, season of the year, feeding practices, and breed type and species. Its concentration in bovine milk is around 30 mg L^{-1}, constituting about 1% of the whey protein. The LPO concentration is low in bovine colostrum, unlike other antibacterial proteins; however, it increases rapidly to reach a maximum at 3–5 days post-partum. Varying levels of LPO activity have been reported in bovine, caprine, ovine, buffalo, camel, human and guinea pig milk (**Table 1**). Bovine milk contains on the average 2.3 U mL^{-1} LPO and is about 20 times richer in peroxidase activity than human milk. Human

Table 1 Lactoperoxidase activity in milk of different mammalian species

Type of milk	*LPO (U mL^{-1})*
Cow	1.5–2.7
Goat	0.04–9.28
Ewe	0.14–3.46
Buffalo	0.9–7.3
Camel	2.2
Human	0.06–0.97
Guinea pig	22
Threshold for antibacterial activity	0.02

colostrum has a high level of MP and a low level of LPO. However, LPO is the principal peroxidase in mature human milk. LPO activities of 0.79 and 4.5 U mL^{-1} have been reported for Saanen goat milk and Creole goat milk, respectively.

Lactoperoxidase System

The LPO system is an antimicrobial system and has been recommended as an alternative to chilling for the preservation of raw milk, especially where lack of capital, unreliable energy supply, and high ambient temperatures make chilling of raw milk practically unattainable. The LPO system has many applications as a natural preservative in the food industry; however, its use has been generally recommended for the dairy industry. Unlike pasteurization and fermentation, the LPO system does not render raw milk safer for consumption; it preserves the initial quality of the product. Since LPO retains its activity at pasteurization temperatures applied to milk, it can be used in combination with heat treatment for the preservation of milk and milk products.

LPO catalyzes the oxidation of thiocyanate (SCN^-) by hydrogen peroxide to yield thiocyanogen $(SCN)_2$, which is then hydrolyzed to hypocyanous acid (HOSCN) or hypothiocyanate (OSCN). OSCN is the major intermediate oxidation product of the LPO-catalyzed oxidation of SCN. Other short-lived intermediates that can be found in varying amounts are thiocyanogen $(SCN)_2$, cyanogen thiocyanate (NC–SCN), cyanosulfurous acid (HO_2SCN), and cyanosulfuric acid (HO_3SCN). Hypocyanous acid and hypothiocyanate are highly reactive oxidizing agents. They react with the sulfhydryl groups and reduce nicotinamide nucleotides of microbial cells. The oxidation of these cellular components, cytoplasmic membranes, carbohydrate and amino acid transport systems, and glycolytic pathways are impaired.

$$SCN^- + H_2O_2 + 2H \xrightarrow{\text{Lactoperoxidase}} (SCN)_2 + 2H_2O$$

$$(SCN)_2 + H_2O \rightarrow OSCN + SCN^- + H$$

$$OSCN \leftrightarrow H + OSCN$$

Thiocyanate can also be directly oxidized to hypothiocyanate:

$$SCN^- + H_2O_2 \xrightarrow{\text{Lactoperoxidase}} OSCN^- + H_2O$$

Apart from the LPO enzyme, exogenous thiocyanate and hydrogen peroxide are needed for a complete and functional LPO system. Thiocyanate (SCN^-) is widely distributed in animal tissues and secretions. It is present in the mammary, salivary, and thyroid glands and their secretions, in organs such as the stomach and kidney, and in fluids such as synovial, cerebral, cervical and spinal fluids, lymph, and plasma. Thiocyanate is found naturally in milk at levels of ~24 mg kg^{-1} in bovine milk, ~18 mg kg^{-1} in ewe's milk, and ~10 mg kg^{-1} in goat milk. The thiocyanate level found in milk varies with breed type, species, feed, and udder health of the animal and also varies with the season of milking. SCN^- is usually present in milk at sufficient concentrations to serve as the principal electron donor in the enzymatic reaction. The thiocyanate level required for activation of the LPO system is 15 mg kg^{-1}. The addition of an exogenous source of thiocyanate, at a concentration of 100–800 mg kg^{-1}, to fully activate the LPO system will enhance the antibacterial effect of the system. Hydrogen peroxide is one of the inorganic peroxide compounds and a strong oxidizing agent and exhibits varying degrees of antimicrobial activity. Unlike thiocyanate, hydrogen peroxide is not detected in milk under normal conditions. However, it can be generated endogenously by polymorphonuclear leukocytes during phagocytosis or by catalase-negative lactic acid bacteria (LAB) such as lactococci, lactobacilli, and streptococci during growth under aerobic conditions. A hydrogen peroxide generating system, for example, sodium percarbonate, is said to be more effective than hydrogen peroxide as a component of the LPO antimicrobial system. This system is 50–100 times more effective than H_2O_2 alone. Hydrogen peroxide has been found to be highly toxic for mammalian cells; however, at low levels of hydrogen peroxide (1000 μmol L^{-1}) and in the presence of LPO and SCN^-, mammalian cells are protected from the toxicity of hydrogen peroxide. A recent report suggests that lactose oxidase (LO) could be used as a novel activator of the LPO system as an alternative to H_2O_2. Lactose oxidase oxidizes lactose and produces H_2O_2 needed for the activation of the LPO system. Activation of the LPO system using LO as an alternative source of H_2O_2 significantly reduced the total bacterial count after 24 h of storage compared with the control and caused greater reductions of *Pseudomonas fragi* in a model system.

Mechanisms of the Antimicrobial Action of Lactoperoxidase System

The antimicrobial effect of the LPO system stems from the reaction of unstable hypothiocyanite with sulfhydryl groups in cell membrane proteins and with low-molecular-weight components of cytoplasmic thiols forming disulfides, sulfenyl thiocyanates, or sulfenic acids. The oxidation of sulfhydryl (SH) groups in microbial enzymes and other proteins is considered to be the key to the antimicrobial action of the LPO system.

$$\text{protein} - SH + OSCN^- \rightarrow \text{protein} - S - SCN + OH^-$$

$$\text{protein} - \text{SH} + (\text{SCN})_2 \rightarrow \text{protein} - \text{S} - \text{SCN} + \text{SCN}^- + \text{H}^+$$

$$\text{protein} - \text{S} - \text{SCN} + H_2\text{O} \rightleftharpoons \text{protein} - \text{S} - \text{OH} + \text{SCN}^- + \text{H}^+$$

The products HOSCN and $OSCN^-$ react rapidly with protein sulfhydryl groups to yield sulfenyl thiocyanates (R–S—SCN). At low concentrations of sulfhydryls (R′—SH), the R–S—SCN may react to form mixed disulfides (R–S—S-R′). At higher concentrations of R′—SH, the R–S—SCN may be reduced back to R—SH. The R–S—SCN may also be hydrolyzed to sulfenic acids (R–S—OH). LPO catalyzes the incorporation of SCN^- into protein substrates. The reaction of $(SCN)_2$ or $OSCN^-$ with proteins oxidizes the protein sulfhydryls to sulfenyl thiocyanate derivatives. Sulfenyl thiocyanate derivatives can undergo further modifications, including reversible hydrolysis, to yield sulfenic acids.

These reactions inhibit bacterial enzymes responsible for respiration and metabolism, notably enzymes having cysteine residues in their active sites. At pH <5, HOSCN inhibits microorganisms by entering the cells as undissociated acid. In the cytoplasm of the microbial cell, the equilibrium favors the undissociated acid; analogous to the antibacterial organic acid mechanism, the generation of protons inside the cells is responsible for the antibacterial activity of LPO at low pH. LPO in milk exhibits an activity maximum at pH 5 and combined with elevated HOSCN concentrations, LPO system has optimal antimicrobial activity. The cytoplasmic membrane or the cytoplasm is the major target of the LPO system's antimicrobial products. The structural damage of microbial cytoplasmic membranes by the oxidation of SH groups results in leakage of potassium ions, amino acids, and polypeptides. Subsequently, uptake of glucose, amino acids, purines, and pyrimidines in the cell and the synthesis of proteins, DNA, and RNA are also inhibited. Cessation of essential cell functions, mainly respiration and metabolism, results in eventual cell death. The cell wall and the membrane may partially limit accessibility of the LPO system's products into the cell but do not exclude it completely.

The stability of hypothiocyanite, $OSCN^-$, is affected by many factors, such as pH, light, metals (Fe, Ni, Cu, Mn), glycerol, and ammonium sulfate as well as by the presence and removal of LPO; however, it is very heat stable.

The antimicrobial activity can be inhibited by reducing agents containing sulfhydryl (SH) groups, such as cysteine, glutathione, mercaptoethanol, dithiothreitol, and sodium hydrosulfite, either by direct binding to the heme group of the enzyme or by scavenging thiocyanate ions. Neither HOSCN nor $OSCN^-$ appears to oxidize SH groups of milk proteins, such as β-lactoglobulin.

Antimicrobial Activity of the Lactoperoxidase System

The LPO system can inhibit the growth and metabolism of different species of microorganisms. It is capable of inhibiting viruses, Gram-positive bacteria, Gram-negative bacteria, fungi, mycoplasms, and parasites, and can be applied at ambient temperatures ranging from 15 to 30 °C for 6 h for the preservation of raw milk. LPO may have a bactericidal or bacteriostatic effect against a range of spoilage and pathogenic bacteria that occur in raw milk. Compared to lysozyme, LPO has a much wider antibacterial spectrum because of the lower specificity of its antibacterial mechanism. The susceptibility of microorganisms to the LPO system depends on the state of their growth. The LPO system is also more effective at low cell densities than at high densities. The lower the cell density, the more lethal the effect of the LPO system and the permeability of the cell wall, with rough mutants showing higher susceptibility. The effect of the LPO system on bacteria can be reversible or irreversible, due to stress response of cells, as well as cross-protection. The capacity of cells to recover from inhibition depends on environmental conditions, for example, temperature and pH, and is also strain specific. Bacteria that survive the initial bactericidal activity of the LPO system exhibit an extended lag phase or recovery period. Cross-protection occurs because some stress response systems share the same/overlapping regulatory pathways. Strains may exhibit cross-protection when subjected to combination treatments during processing. Acid-adapted *Escherichia coli* elicits cross-protection against combined low pH (pH 4.0 and 5.0) and activated LPO. Changes in the outer membrane porins (*omp*C and *omp*F) and outer membrane fatty acids contribute to cross-protection. Porin-mediated outer membrane permeability for small hydrophilic molecules leads to increased tolerance to the LPO enzyme system because of the reduced uptake of $OSCN^-$.

The bacteriostatic effect of the LPO system on *Staphylococcus aureus* and its bactericidal effect on enteric pathogens including multiple antibiotic-resistant *E. coli* strains, *Listeria monocytogenes*, *Salmonella typhimurium*, and *Brucella melitensis* in goat milk have been demonstrated. Inhibition of other pathogens such as *Campylobacter jejuni* and vegetative cells of *Bacillus cereus* has been reported. Other bacteria that were found to be inhibited by the LPO system include *Citrobacter freundii*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella enteritidis*, *Salmonella* Typhi, *Vibrio cholerae*, *Helicobacter pylori*, *Streptococcus uberis*, and *S. aureus*. Acid production, oxygen uptake, and consequently H_2O_2 excretion were inhibited in *Streptococcus mutans*, *Streptococcus sanguis*, *Streptococcus mitis*, and *Streptococcus thermophilus*. *Sc. sanguis* and *Sc. mitis* showed more resistance to the LPO system than *Sc. mutans* and *Sc. thermophilus*. This was attributed to the higher activity of NADH-OSCN oxidoreductase in the former strains. The primary target of $OSCN^-$ in the glycolytic pathway was glyceraldehyde 3-phosphate dehydrogenase.

The LPO system plays a role in protecting the lactating mammary gland from infection with *Sc. uberis* and LPO may be used is an index of mastitis infection. Any disease condition that causes an increased concentration of leukocytes in milk will increase the activities of certain enzymes in milk. LPO is synthesized mainly by polymorphonuclear leukocytes and colostrum peroxidase activity increases with an increase in the somatic cell count (SCC) of milk. Although high SCC increases the level of LPO in bovine, goat, and human milk, the correlation between SCC and LPO level is variable.

The LPO–thiocyanate–H_2O_2 system was found to inhibit the growth and proliferation of many fungal and yeast species, such as *Trichoderma* spp. *Corynespora cassiicola*, *Phytophthora meadii*, *Alternaria* spp. *Penicillium chrysogenum*, *Claviceps* spp. and *Corticium*

salmonicolor. *Candida albicans* and *Pythium* spp. were not affected by the LPO system. Different species were inhibited at different concentrations of goat milk LPO, for example, *Aspergillus niger* required a minimum of 475 μg mL^{-1}, whereas *Claviceps* spp. required 62 μg mL^{-1} for total inhibition. LPO has the ability to degrade aflatoxin in the presence of sodium chloride (225 mmol L^{-1}) and H_2O_2 (50 mmol L^{-1}). Comparable amounts of LPO degraded aflatoxin G1 ~1.5 times faster than aflatoxin B1. The rate of degradation of aflatoxin B1 increased from 3.6% to 5.1% per 24 h as the amount of LPO in the reaction mixture increased from 50 to 500 U mL^{-1}.

LPO has been shown to affect both poliovirus and vaccinia virus with halides (I^-, Br^-) as electron donors. LPO and glucose oxidase are virucidal to HIV-1 in the presence of sodium iodide, as assessed by the loss of viral replication in a syncytium-forming assay or by the inhibition of cytopathic effects in infected cells. Recent studies have shown that the LPO system (camel, bovine and human) exhibits antiviral activities against hepatitis C virus genotype 4 and herpes simplex virus Type 1.

Use of the Lactoperoxidase System for Preservation of Raw Milk

The most widely recommended industrial application of the LPO system in food production is in the dairy industry for the preservation of raw milk during storage and/or transportation to processing plants. Antimicrobial agents formed through activation of the LPO system in milk cause inhibition of various spoilage and pathogenic organisms, thus enhancing the microbiological quality of milk. The antibacterial activity of the LPO system in milk against psychrotrophic spoilage organisms has been widely demonstrated. The LPO system was found to be bactericidal against *Pseudomonas fluorescens* and *E. coli* and the bactericidal effect depends on SCN^- and H_2O_2 concentrations and the initial inoculum size. Activation of the LPO system in milk resulted in a substantial reduction of the bacterial flora and prevented the growth of psychrotrophic bacteria for up to 5 days. The LPO-treatment neither altered physico-chemical properties of milk nor developed LPO-resistant bacteria.

The LPO system has been widely used to control mesophilic spoilage bacteria in bovine milk. It has also been used for the preservation of buffalo milk both at ambient and refrigeration temperatures. Similarly, activation of the LPO system was found to be an effective means of preservation against microbial growth in raw ewe and goat milk samples stored at 20 °C or 30 °C for at least 6 h. The length of the antibacterial effect achieved by activation of the LPO system is inversely related to the storage temperature of the milk. When milk is stored at 30, 25, 20 and 15 °C, the antibacterial effect of the LPO system lasts for 7–8, 11–12, 16–17 and 24–26 h, respectively.

Field experiments in developing countries have demonstrated that activation of the LPO system at collection points and during transportation can significantly extend the shelf life and substantially improve the hygienic quality of raw milk at ambient temperature (30 °C).

The LPO system can also be applied in combination with other treatments used to preserve milk. The use of the LPO system in combination with heat treatment has proved effective in eliminating vegetative microorganisms in milk. The activated LPO system in combination with pasteurization extended the shelf-life of cow milk held at 10 °C by more than 20 days, compared to untreated milk. When raw cow milk was LPO-activated prior to pasteurization, an improvement in the quality of pasteurized milk was observed probably due to reduced heat resistance of microorganisms as a result of the LPO-treatment. The LPO system in combination with low pH also reduced *E. coli* and *Shigella* spp. cell numbers to undetectable levels after 24 h exposure in fruit and vegetable juices. Activated LPO may enhance resistance to sublethal heat treatments; therefore, the sequence of application of heat and LPO treatments is important. High-pressure homogenization together with the activated LPO system and lysozyme at 37 °C reduced both *L. monocytogenes* and *E. coli* to undetectable levels. A synergistic effect of LPO and nisin in ultra-high temperature (UHT) skim milk resulted in a decrease in *L. monocytogenes*, and activation of the LPO system greatly enhanced the thermal destruction of *L. monocytogenes* in milk during pasteurization. Whey protein films incorporating the LPO system inhibited *Salmonella enterica* and *E. coli*.

The use of the LPO system provides a reliable and economical alternative for preserving raw milk, particularly in small-scale dairy enterprises when coupled with good hygiene and sanitation. Its economic viability, either as a standalone system or in combination with refrigeration, and its potential to significantly reduce milk losses and thereby increase the amount of milk collected leads to direct benefits to both milk producers and consumers.

Application of the Lactoperoxidase System in the Manufacturing of Fermented Dairy Products

The use of LPO system has the potential to increase the quality and quantity of raw milk available for further processing into dairy products. One of the problems of using the LPO system for the production of fermented dairy products is the effect it has on lactic starter cultures.

The LPO system inhibits the activity of lactic starter cultures resulting in reduced acid production and weaker gels in acid-coagulated dairy products. The biochemical effect that the LPO system has on individual lactic cultures varies with the inherent resistance of LAB cultures and the type of milk used. Exposure to the LPO system causes a general decrease in lactic acid production by thermophilic LAB cultures used for milk fermentation and delays the coagulation of milk. Mesophilic starter cultures have also been shown to exhibit resistance to the LPO system. Phage-resistant mutants can be more susceptible to the LPO system than their respective parent strains.

Single-strain cultures of *Lactococcus lactis* subsp. *lactis*, *Lc. lactis* subsp. *cremoris*, *Lc. lactis* subsp. *lactis* biovar *diacetylactis*, and *Leuconostoc mesenteriodes* subsp. *cremoris*, and probiotic *Bifidobacterium longum* have also been shown to be unaffected by the LPO system. LPO activity depends on the severity of the heat treatment of milk, due to the effect of heat on LPO. Acid production by LAB can be affected by HTST, but can be restored by the addition of LPO. Inhibition of LAB strains in yogurt, *Lactobacillus delbrueckii* subsp. *bulgaricus* and *Sc. thermophilus*, is variable.

Since some strains of lactic acid bacteria are less affected and resistant to the LPO system, it is now possible to use the LPO system to improve the quality and safety of various fermented dairy products. Acceptable varieties of soft and hard cheeses (Cheddar, Saint-Paulin, cottage and fresh type cheeses) can be made from LPO-treated cow milk. Activation of the LPO system significantly increased the yield of fresh type cheese made from cow milk and it resulted in cheese with acceptable pH and appearance and microbiological counts. Similarly, treatment of buffalo milk with LPO and H_2O_2 increased yield and improved the organoleptic quality of the cheese. Cheddar cheese made from milk treated with a combination of microfiltration and activation of the LPO system had similar characteristics as untreated cheese. An activated LPO system can effectively reduce the numbers of pseudomonads, *E. coli* and *Salmonella thyphimurium* in cottage cheese without adverse effect on the pH or sensory characteristic of the cheese. Thus, it functions as an effective natural preservation system to increase the safety and stability of cottage cheese. Moreover, LPO-treatment was found to eliminate *L. monocytogenes* from the surface of French soft cheese. Saint-Paulin cheese made from LPO-treated cow milk was found to have significantly lower coliform and yeast and mold counts than the untreated control and prevented excessive proteolysis of the cheese caused by proteinases of Gram negative psychrotrophs. Combination of LPO system and lysozyme was able to significantly reduce the total microbial count in Dangke (Indonesian soft milk cheese) by a factor of 2.48 log cycles during 8 h of storage at room temperature. A weak body and extended curdling time was observed in Turkish white-brined cheese (Beyaz peynir) made from LPO system-activated cow's milk. It was also found that the time needed for reaching the stretching stage in Mozzarella cheese was extended up to 2 h when cheese was produced from the LPO system activated buffalo's milk.

Preservation of goat milk by the LPO system was found to improve the microbiological quality and flavor of goat milk Gouda cheese without any detrimental effect to the chemical composition of the cheese. LPO-treatment significantly reduce coliform and coagulase positive staphylococci during ripening of goat milk Gouda cheese. Moreover, it reduces the level of lipolysis in Gouda cheese without affecting proteolysis in the cheese during the ripening period. Activation of the LPO system in ewe milk was used to prevent excessive proteolysis and softening of Manchego cheese texture caused by proteinases of Gram-negative psychrotrophs.

Fermented milk products such as yoghurt, acidophilus milk and Dahi can be successfully made from LPO-treated milk. LPO-treatment did not adversely affect the quality of Dahi, yoghurt and acidophilus milk made from cow milk or buffalo milk, but a slow rate of acid production was encountered while using LPO-activated milk. Activation of the LPO system was reported to suppress excessive acid production of yoghurt during refrigerated storage and reduces hardness and apparent viscosity of yoghurt.

The LPO-system does not induce adverse effects on the chemical, physical or sensory characteristics of raw milk and processed dairy products. Therefore, LPO system is an efficient alternative for preservation of raw milk that will be subjected to further processing. Since heat treatment is more effective if the initial microbial cell counts are minimized before processing, application of the LPO prior to heating provides a complementary and synergistic effect in reducing microbial load in milk. That is, activation of the LPO system followed by heating can increase the margin of safety with respect to milk-borne pathogens.

The LPO-system can be used alone when refrigeration is not available, or in synergy with cooling/chilling and can be considered to be an efficient tool to improve the quality and quantity of milk and dairy products by maintaining the microbiological quality of raw milk.

The LPO system should be considered as suitable to extend milk collection distances particularly in developing countries and thereby increase the amount of milk available for further processing and subsequent marketing. It can be used to improve the quality of processed products because of its proven bacteriostatic effect from milk collection to final processing.

See Also: BACTERIA, BENEFICIAL: Probiotic Lactic Acid Bacteria: An Overview; Brucella spp.; Cheese: Public Health Aspects; Escherichia coli; Fermented Milks: Starter Cultures; Listeria monocytogenes; Non-Thermal Treatment of Milk: High Pressure Processing; Psychrotrophic Bacteria: Pseudomonas spp.; Raw Milk Cheeses; Salmonella spp; Staphylococcus aureus - Molecular; Yeasts in Milk and Dairy Products

Further Reading

Al-Baarri, A.M., Legowo, A.M., Arum, S.K., Hayakawa, S., 2018. Extending Shelf Life of Indonesian Soft Milk Cheese (Dangke) by Lactoperoxidase System and Lysozyme. Int. J. Food Sci. 2018 https://doi.org/10.1155/2018/4305395. Article ID 4305395.

Atasever, A., Ozdemir, H., Gulcin, I., Kufrevioglu, O.I., 2013. One-step purification of lactoperoxidase from bovine milk by affinity chromatography. Food Chem. 136 (2), 864–870.

Bafort, F., Parisi, O., Perraudin, J.P., Jijakli, M.H., 2014. Mode of action of lactoperoxidase as related to its antimicrobial activity: a review. Enzym. Res. 1–13.

Boulares, M., Mankai, M.L., Hassouna, M., 2011. Effect of activating lactoperoxidase system in cheese milk on the quality of Saint-Paulin cheese. Int. J. Dairy Technol. 64 (1), 75–83.

De Spiegeleer, P., Sermon, J., Vanoirbeek, K., Aertsen, A., Michiels, C.W., 2005. Role of porins in sensitivity of *Escherichia coli* to antibacterial activity of the lactoperoxidase enzyme system. Appl. Environ. Microbiol. 71, 3512–3518.

Dumitraşcu, L., Stănciuc, N., Stanciu, S., Râpeanu, G., 2012. Thermal inactivation of lactoperoxidase in goat, sheep and bovine milk - a comparative kinetic and thermodynamic study. J. Food Eng. 113 (1), 47–52.

El-Fakharany, E., Uversky, V., Redwan, E., 2017. Comparative analysis of the antiviral activity of camel, bovine, and human lactoperoxidases against herpes simplex virus type 1 Part A. Appl. Biochem. Biotechnol. 182 (1), 294–310.

FAO/WHO, 2006. Benefits and potential risks of the lactoperoxidase system of raw milk preservation: report of an FAO/WHO technical meeting, FAO Headquarters, Rome, Italy, 28 November - 2 December 2005. Food and Agriculture Organization of the United Nations/World Health Organization, Rome/Geneva.

Fox, P.F., Kelly, A.L., 2006. Indigenous enzymes in milk: overview and historical aspects – Part 1. Int. Dairy J. 16, 500–516.

IDF, 1988. Code of practices for the preservation of raw milk by the lactoperoxidase system. Bull. Int. Dairy Feder. 234, 1–15.

Kussengrager, K.D., van Hooijdonk, A.C.M., 2000. Lactoperoxidase: physico-chemical properties, occurrence, mechanism of action and applications. Br. J. Nutr. 84, S19–S25.

Lara-Aguilar, S., Alcaine, S.D., 2018. Lactose oxidase: A novel activator of the lactoperoxidase system in milk for improved shelf life. J. Dairy Sci. 102, 1–10.

Lorenzen, P.C., Wernery, R., Johnson, B., Jose, S., Wernery, U., 2011. Evaluation of indigenous enzyme activities in raw and pasteurized camel milk. Small Rum. Rese. 97, 79–82.

Mal, G., Pathak, K.M.L., 2010. Camel milk and milk products. SMVS' Dairy Year Book. National Research Centre on Camel, India, pp. 97–103.

Özer, B., 2014. Lactoperoxidase and lactoferrin. In: Batt, C.A., Tortorello, M.L. (Eds.), Encyclopedia of Food Microbiology, Second Edition, Volume 2. Elsevier Ltd, pp. 930–935.

Parry-Hanson, A., Jooste, P.J., Buys, E.M., 2009. The influence of lactoperoxidase, heat and low pH on survival of acid-adapted and non-adapted *Escherichia coli* O157:H7 in goat milk. Int. Dairy J. 19, 417–421.

Seifu, E., Buys, E.M., Donkin, E.F., 2005. Significance of the lactoperoxidase system in the dairy industry and its potential applications: a review. Trends Food Sci. Technol. 16 (4), 137–154.

Tayefi-Nasrabadi, H., Hoseinpour-fayzi, M.A., Mohasseli, M., 2011. Effect of heat treatment on lactoperoxidase activity in camel milk: A comparison with bovine lactoperoxidase. Small Rum. Res. 99, 87–190.

Wernery, U., Maier, U., Johnson, B., George, R.M., Braun, F., 2006. Com parative study on different enzymes evaluating heat treatment of dromedary milk. Milchwissen 61, 281–285.